The Veterinary Journal 225 (2017) 63–68

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Original Article

A quantitative swab is a good non-invasive alternative to a quantitative biopsy for quantifying bacterial load in wounds healing by second intention in horses

L.L. Van Hecke[a,*], K. Hermans[b], M. Haspeslagh[a], K. Chiers[b], E. Pint[a], F. Boyen[b], A.M. Martens[a]

[a] Department of Surgery and Anaesthesiology of Domestic Animals, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
[b] Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ARTICLE INFO

Article history:
Accepted 16 June 2017

Keywords:
Diagnosis
Horse
Infection
Second intention
Wound

ABSTRACT

The aim of this study was to evaluate different techniques for diagnosing wound infection in wounds healing by second intention in horses and to assess the effect of a vortex and sonication protocol on quantitative bacteriology in specimens with a histologically confirmed biofilm. In 50 wounds healing by second intention, a clinical assessment, a quantitative swab, a semi-quantitative swab, and a swab for cytology were compared to a quantitative tissue biopsy (reference standard). Part of the biopsy specimen was examined histologically for evidence of a biofilm.

There was a significant, high correlation ($P < 0.001$; $r = 0.747$) between the outcome of the quantitative swabs and the quantitative biopsies. The semi-quantitative swabs showed a significant, moderate correlation with the quantitative biopsies ($P < 0.001$; $\rho = 0.524$). Higher white blood cell counts for cytology were significantly associated with lower $\log_{10}$ colony-forming units (CFU) in the wounds ($P = 0.02$). Wounds with black granulation tissue showed significantly higher $\log_{10}$ CFU ($P = 0.003$). Specimens with biofilms did not yield higher bacteriological counts after a vortex and sonication protocol was performed to release bacteria from the biofilm. Based on these findings, a quantitative swab is an acceptable non-invasive alternative to a quantitative biopsy for quantifying bacterial load in equine wounds healing by second intention.

© 2017 Elsevier Ltd. All rights reserved.

Introduction

In horses, traumatic wounds are always contaminated with micro-organisms and can result in significant morbidity (Provost, 2012; Theoret et al., 2016). This contamination can evolve towards an infection depending on the virulence of the micro-organisms, their load, the nature of the wound, and the host's immune response (Sibbald et al., 2006). Since infection retards wound healing, early detection is of utmost importance (Stashak, 2008a).

In human medicine, a quantitative tissue biopsy is considered the reference standard method for diagnosing wound infection (Robson, 1997; Ratliff and Rodeheaver, 2002; Dow, 2003; Serena et al. 2006; Bonham, 2009). The presence of $>10^5$ colony-forming units (CFU)/g tissue is generally accepted as being indicative of infection (Robson, 1997; Bowler et al., 2001; Rondas et al., 2013); the validity of this criterion has been investigated in horses (Peyton and Connelly, 1983). Several human medical studies have demonstrated more practical and less invasive alternatives (Levine et al., 1976; Bowler et al., 2001; Gardner et al., 2001; Ratliff and Rodeheaver, 2002; Gardner et al. 2006; Serena et al., 2006; Davies et al., 2007; Woo and Sibbald, 2009).

In equine medicine, the 10^5 CFU/g tissue criterion, in combination with qualitative bacteriology, are recommended for diagnosing infection in traumatic wounds (Hendrickson and Virgin, 2005; Stashak, 2008b; Provost, 2012). Nevertheless, in equine practice, wound infection is generally diagnosed based purely on clinical signs. However, to the authors' knowledge, studies investigating which clinical signs are indicative of a high bacterial load in equine wounds are lacking.

Wound infection in horses has been associated with biofilms (Freeman et al., 2009). Biofilms can delay wound healing because bacteria in biofilms have enhanced virulence, are protected from

* Corresponding author.
E-mail address: lore.vanhecke@ugent.be (L.L. Van Hecke).

http://dx.doi.org/10.1016/j.tvjl.2017.06.004
1090-0233/© 2017 Elsevier Ltd. All rights reserved.

the immune response of the host, and are more likely to be resistant to antimicrobials. The bacteria are embedded in an extracellular polymeric substance and are in a slow-growing or non-growing state (Freeman et al., 2009). Therefore, some of these bacteria might remain undetected during qualitative and quantitative bacteriological analysis. However, the impact of a biofilm-disrupting protocol on the bacterial load of equine wound specimens has not been investigated.

The goal of this study was to correlate a clinical assessment, a quantitative swab, a semi-quantitative swab and a swab for cytology with the bacterial load of an open wound determined by quantitative biopsy in horses. Additionally, the impact of a biofilm-disrupting protocol on the quantification of bacterial load in wounds with histologically confirmed biofilms was tested. Our hypothesis was that both a quantitative and semi-quantitative swab would be valid non-invasive alternatives to a quantitative tissue biopsy for quantifying the bacterial load in equine wounds healing by second intention. Additionally, we hypothesised that bacterial counts would increase after applying a biofilm-disrupting protocol to wound specimens with a histologically confirmed biofilm.

Materials and methods

Following ethical committee approval (Number 2013-65; Approval date 8 May 2013), horses admitted to the Faculty of Veterinary Medicine (Ghent University) were screened for inclusion in this study (May 2013 to February 2015). Inclusion criteria were as follows: one or multiple traumatic wounds healing by second intention with surface area of $\geq$4 cm^2 and with granulation tissue. Horses with a systemic condition (e.g. Cushing's syndrome) or receiving medication (e.g. glucocorticoids) that could affect the immune system were excluded. Owners signed an informed consent before commencement of the study.

Wound specimens

Relevant data from each horse (age, presence of fever > 38.5 °C) and of the wound (location and wound type) were recorded. The first author performed the evaluation and sampling of the wound during a bandage or cast change.

The wound was evaluated for clinical signs of infection using a checklist (Table 1). The wound pH was measured using a pH stick (Panpeha No. 112, Novolab), which was placed on the wound surface until it was completely soaked with wound fluid.

After the clinical assessment, three swabs were taken using the method described by Levine et al. (1976). First, the wound was rinsed with sterile saline solution (0.9% NaCl in a 35 mL syringe with a 19 G needle) to eliminate surface contamination. Excess fluid was removed by gently blotting the wound surface with dry sterile gauze. A sterile rayon-tipped swab was moistened with a few drops of sterile saline solution (0.9% NaCl) and a sterile template of 1 cm^2 was placed on the approximate centre of the wound. Subsequently, a swab was taken over the 1 cm^2 area over 5 s while rotating the swab between the thumb and the index finger over 360° with sufficient pressure to express tissue fluid. The swab was put into a sterile container without transport medium and labelled with a reference number and the date. The other two swabs were taken using the same procedure, but the 1 cm^2 template was moved slightly to avoid swabbing the same area twice. The swabs were processed within 1 h or placed into a refrigerator (4 °C) for a maximum of 6 h.

Finally, a wound specimen was taken using a 6 mm diameter punch biopsy tool, which was placed near the centre of the wound over viable tissue while avoiding the areas that had been swabbed. The underside of the biopsy was marked with sterile Indian ink and placed into a sterile container labelled with a reference number and the date. The biopsies were processed within 1 h or placed into a refrigerator (4 °C) for a maximum of 6 h.

Swab specimen processing

One swab was processed using quantitative and semi-quantitative bacteriological procedures. A second swab underwent a vortex and sonication protocol before being processed for quantitative and semi-quantitative procedures. The third swab was processed for cytology. The techniques are described in detail below.

The rayon swab tip used for the quantitative bacteriological data was cut with sterile scissors and put into a 1.5 mL Eppendorf tube filled with 1 mL of sterile phosphate buffered saline (PBS). The tip was vortexed for 30 s and then serially diluted 10-fold in PBS. Next, 20 μL aliquots of each dilution were spot-plated onto a Columbia agar supplemented with 5% sheep blood (Oxoid). The Columbia agar plates were incubated in a 5% CO_2-enriched atmosphere at 37 °C for 24 and 48 h, after which the colony-forming units (CFU) were counted. The number of CFU/swab was then calculated taking into account the dilution.

The same swab tip was used to provide semi-quantitative bacteriological data. Twenty μL of the undiluted solution containing the swab was spotted and streaked out on the first quadrant of a Columbia agar plate with 5% sheep blood (Oxoid). Next, the other three quadrants were streaked out, each time using a sterile inoculation loop and crossing the inoculation lines of the former quadrant twice, thus serially diluting the original inoculation spot. The plate was incubated as described above, after which the bacterial growth in the quadrants was assessed. The bacterial burden was classified as scant (+), light (++), moderate (+++), or heavy (++++) depending on the presence of growth in the first, second, third or fourth quadrant, respectively.

The rayon tip of the swab used to assess the effect of a biofilm-disrupting protocol was also cut off with sterile scissors and put into a 1.5 mL Eppendorf tube filled with 1 mL of sterile PBS. Next, the tip was vortexed for 30 s and then sonicated 30 s in a sonication bath (B5210, 47 kHz, Branson). This was repeated twice to release bacteria from a potential biofilm (Brackman et al., 2013). Subsequently, the swab was processed for quantitative and semi-quantitative bacteriology as described above.

The third swab was processed for cytology. The swab was placed centrally on a glass slide and rolled back and forward, so the middle third of the glass was covered with a thin layer of wound exudate. Subsequently, the slide was air dried and Gram-stained. The cytology specimens were examined under a light microscope (CX31, Olympus) at magnification 1000× with immersion oil. The number of white blood cells was counted in 10 1000× high power fields (HPFs). Mean white blood cells/HPF was then calculated.

Tissue specimen processing

The tissue biopsy was cut aseptically into three pieces. One part was fixed in 4% formaldehyde for histology. The other two pieces were weighed aseptically, put into a 1.5 mL microcentrifuge tube filled with 1 mL of sterile PBS, and homogenised with a disposable tissue grinder (disposable pellet mixer 1.5 mL, VWR). One piece was vortexed for 30 s, and the other one underwent the vortex and sonication protocol described for the swab specimens. Both tissue solutions were serially diluted 10-fold in PBS and each dilution was spot-plated on Columbia agar with 5% sheep blood (Oxoid). The plates were incubated as described above, after which the CFU were counted. This number was converted to CFU/g tissue by taking the dilution and the mass of the biopsy into account.

Histology

The formalin-fixed tissue specimen was embedded in paraffin, sectioned in 4 μm slices, and stained with four histological stains: haematoxylin and eosin stain (HE), Gram stain, Giemsa stain, and periodic acid shiff (PAS) stain. The stained

Table 1
Checklist and clinical variables used to evaluate wounds for infection.

Variables	Presence
Exudate (oozed through the third layer of the bandage)	Clear/sanguineous/purulent
Red granulation tissue (beefy aspect)	Present/absent
Yellow necrosis/slough/discolouration of granulation tissue	Present/absent
Black necrosis/slough/discolouration of granulation tissue	Present/absent
Exuberant granulation tissue	Present/absent
Oedematous granulation tissue (glassy, shiny aspect)	Present/absent
Friable granulation tissue (bled easily when probing wound surface and base)	Present/absent
Bone visible or felt with a probe	Present/absent
Oedema	Around the wound/part of the limb/entire limb
Unpleasant odour	Present/absent
Pain (difficult to touch the wound even when the horse is sedated)	Present/absent

Download English Version:

https://daneshyari.com/en/article/5544882

Download Persian Version:

https://daneshyari.com/article/5544882

Daneshyari.com